Some Chemical Aspects of Polychlorinated Biphenyls (PCBs)

by J. William Cook*

In the past few years considerable scientific information has appeared on the chemistry and biological effects of the PCBs and related materials. The amount of chemical investigation, and especially the biological effects investigation that can be done on these products, is almost limitless. Even though the PCBs show a great deal of inertness, a property very useful in their industrial applications, they certainly do change in most instances before they are inadvertently consumed by man. It is important that we learn more about the nature of these products and the changes they undergo. I believe that the choice of scientific investigation that will provide effective answers is going to be made by close cooperation between the scientists in the field of chemistry and those in the various biological disciplines. I hope that conferences like this one will help give us guidance as to the most fruitful avenues to follow in research and will assist in the interpretation of the data obtained. It is simpler to acquire data than to interpret them.

No attempt has been made to make or present a complete survey of literature on chemistry of PCBs. Instead, I have chosen to discuss in a general way some facets of the chemistry which I think are interesting and relevant to the subject of this conference. For the most part I have drawn upon illustrations from our FDA laboratories because of the easily available material.

There are a number of statements in the literature and the public press which liken PCBs to DDTs. If this were the case, we would need to do little research in chemistry of PCBs, because the chemistry of the DDTs and related compounds is well known. Even so, all the biological effects of DDTs are by no means known or fully understood even though there has been much work during the past 25 years.

In contrast to the three well known isomers of DDT—that is, the p,p′, o,p′, and o,o′-dichloro-compounds, the term polychlorobiphenyls or PCBs encompasses a very complex heterogeneous series of chemicals. When manufactured in a certain way, they form technical products which have enjoyed tremendous industrial use over the past 40 years because of the unique chemical and physical properties of the complex mixtures.

The exact chemical composition of these mixtures has not been determined. There probably was no incentive to determine the real nature of all the components as far as the industrial value of the products was concerned, but now that it has been found that there are untoward biological effects from these products and that their residues are widely distributed, there is need for more knowledge than is now available.

There are also statements in the literature which in effect assert that PCB analytical methods and limits for residues should apply to specific chemicals within the series rather than to PCB undefined. Stated differently, if the toxicity of the PCBs is mainly due to certain compounds in the mixtures, then we could be justified in developing methodology to separate, quantitate, and evaluate these toxic compounds. We will need to know more about the chemistry and biological effects before we can be so specific.

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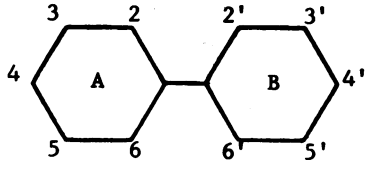
Until we have developed data which, on the one hand, show that essentially all components have about the same biological effects or, on the other hand, show that some are greatly different from others, we have no real scientific basis on which to make a choice. Of course there is evidence that some industrial PCB products contain polychlorodibenzofurans. We need to know how and where these form. Do they form somehow in the environment, and if so, do they survive through the food chain to man? If so, are there great differences in biological effects among the large number of possible polychlorofurans as is the case among the polychlorodioxins? If these compounds are present in foods, we certainly should try to identify them soon, but if they are like the dioxins, they will be difficult to measure in food.

PCBs are manufactured in France, Germany, Great Britain, Italy, Japan, Russia and U.S.A.

Assuming that the starting raw product is only biphenyl, and assuming that no reaction takes place except chlorination of biphenyl during manufacture, 210 different chemicals are theoretically possible from the 10 positions where chlorine can add on as shown in Fig. 1.



Chlorines on B ring	Chlorines on A ring: 0	1	2	3	4	5
0	1	3	6	6	3	1
1		6	18	18	9	3
2			21	36	18	6
3				21	18	6
4					6	3
5						1

FIGURE 1. Structural formula for biphenyl and theoretically possible sites for chlorination.

It is not certain how many of these do appear in significant amounts in the technical products or in food. It is possible that a few molecules of each is present in each of the technical products.

It is important to point out that, in the industrial products, the PCBs are associated with chloroterphenyls. There are three possible terphenyls (the o, m, and p) as starting materials in contrast to one biphenyl as a starting material, and also there are 14 possible points instead of 10 at which each of the 3 isomers may be chlorinated. Therefore, these compounds could result in a considerably more complex mixture than the PCBs. The chloroterphenyls can be associated with the PCBs in the ecosystem, and they are not readily determined by the usual methods of analysis for PCBs and the pesticides. Later some gas chromatograms will be shown that illustrate some of the relationships.

The industrially important products are formed by chlorination of biphenyl to a given chlorine content. Products which contain 21, 42, 48, 54 and 60% chlorine by weight appear to be the most important ones. The end point of the desired average chlorine content is found by making specific gravity or softening point measurements on samples withdrawn during chlorination. The crude products so formed are generally purified to remove color and traces of hydrogen chloride and ferric chloride, largely by distillation. Mixing of distillate is also used to obtain uniform material of the desired composition (1).

Various factors during the manufacturing process can affect the proportion of the various isomers and the proportion of compounds of higher or lower chlorine content than the average (1). Therefore products manufactured by different processes could yield products which give somewhat different amounts of various components. If some components have different biological effects than others, then different samples would produce unequal effects.

Figure 2 shows gas chromatograms of two PCBs; A is Aroclor 1221, and B is 1232. These and the following gas chromatograms (Figs. 3–8) were made by the conditions used in FDA laboratories. The patterns generally look like those we see from other laboratories, so the products seem to be relatively uniform, except for Aroclor 1232. This appears not to have been a fully consistent product in the past.

Figure 3 shows more in the series. A is 1242, and B is 1248.

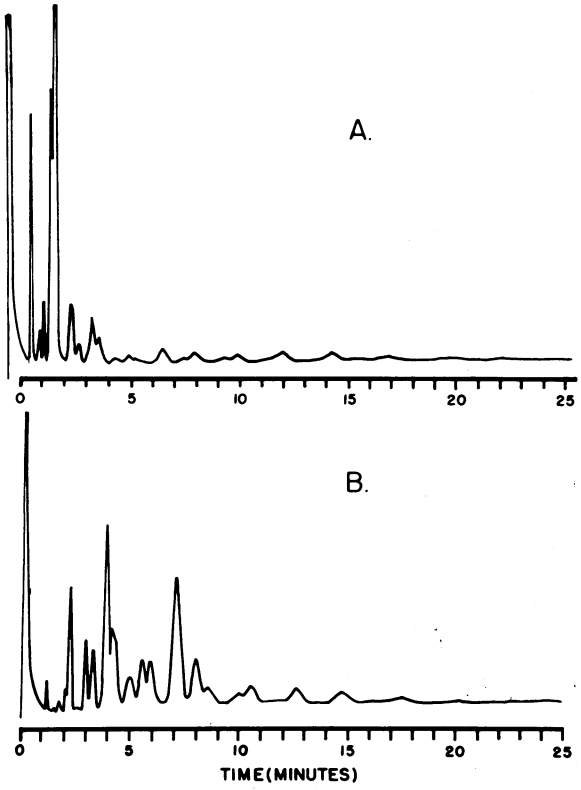


FIGURE 2. Gas chromatograms of PCBs. A is Aroclor 1221. B is 1232.

Figure 4 shows three more in the series; A is 1254, B is 1260, and C is 1262. Note that there is an overlap of peaks. This does not prove that they always represent the same compounds, but in all probability the major part of the peaks is the same. Those which appear in the lower series probably would have been chlorinated to higher chlorine content if the product had stayed in the reaction chamber longer. Generally, as the peaks drop off on the left of the chromatograms, the ones on the right increase, or new ones appear. In all probability there are a few molecules of every possible structure present in each product. Only the predominant ones are in high enough concentrations to give the detector responses. Also note that the time the PCBs are in the gas

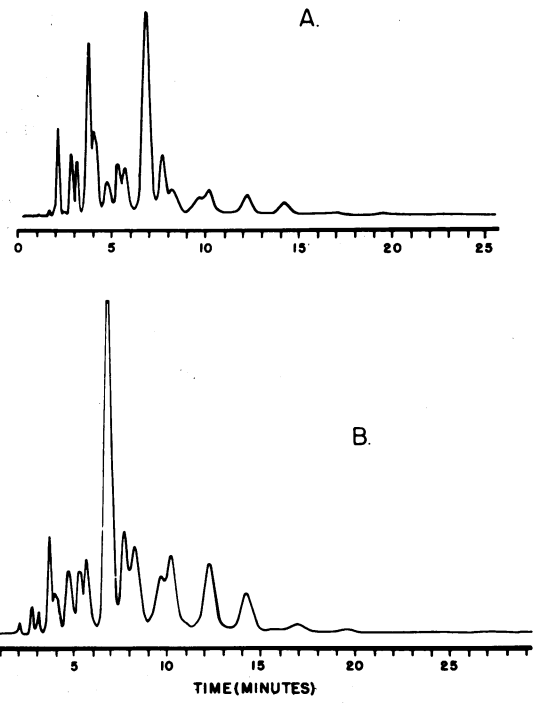


FIGURE 3. Gas chromatograms of PCBs. A is 1242 and B is 1248.

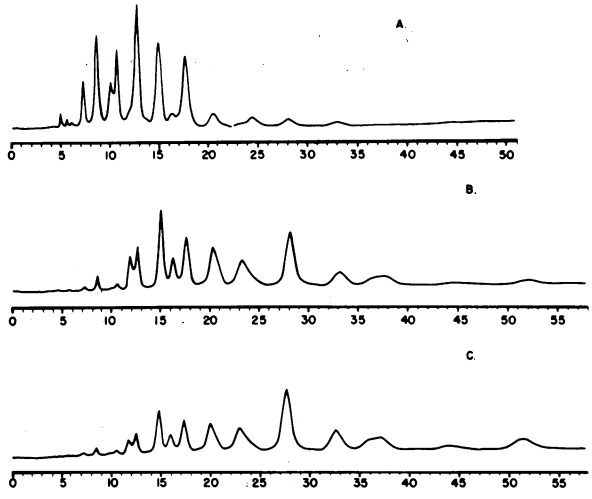


FIGURE 4. Gas chromatograms of PCBs. A is 1254, B is 1260 and C is 1262.

chromatograph under these conditions is between 0 and 50 minutes.

Figure 5 shows a mixed biphenylterphenyl product (Aroclor 4465); note that some come out early, and some come out late. Some of the early ones look like 1260 and 1262 in Fig. 4.

Figure 6 shows a polychloroterphenyl-(Aroclor 5442). Note that there are peaks which come out as early as portions of Aroclor 1242 and 1248. The proportion of the early eluters is relatively low; however, if accumulation through the food chain occurred, they may appear as PCBs.

Figure 7 shows a comparison between the gas chromatograms of Kanechlor 400 from Japan and Aroclor 1248 from U.S.A. This is presented to show that the two products look remarkably alike, even though one was made across the sea. The one from Japan may be manufactured by the same processes as the U.S. one.

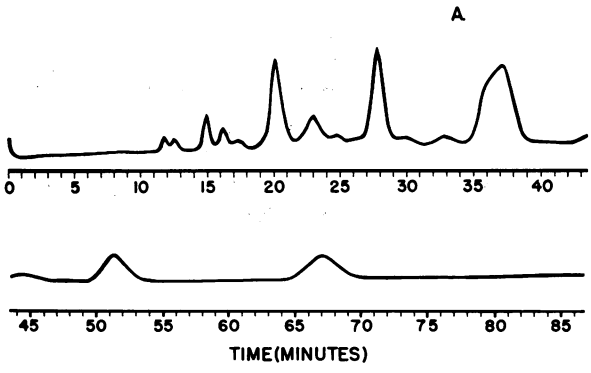


FIGURE 5. Gas chromatogram of a mixed biphenyl-terphenyl product (Aroclor 4465).

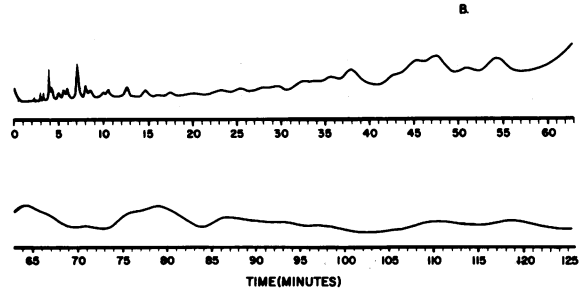


FIGURE 6. Gas chromatogram of a polychloroterphenyl (Aroclor 5442).

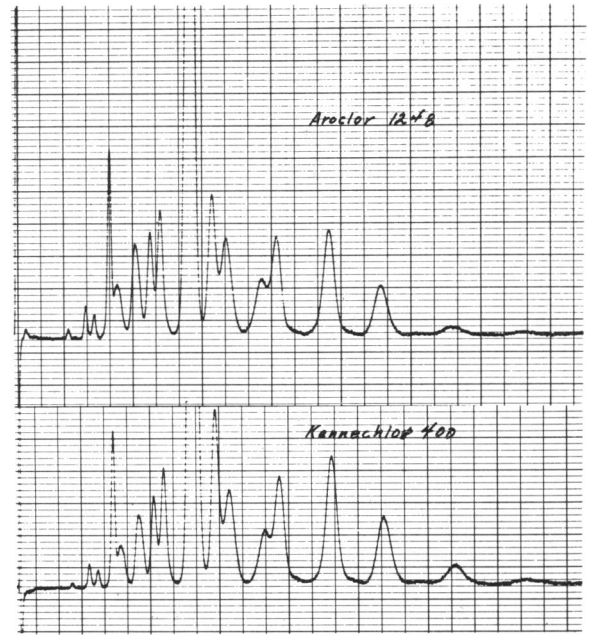


FIGURE 7. Comparison of gas chromatograms of Kanechlor 400 (Japan) and Aroclor 1248 (USA).

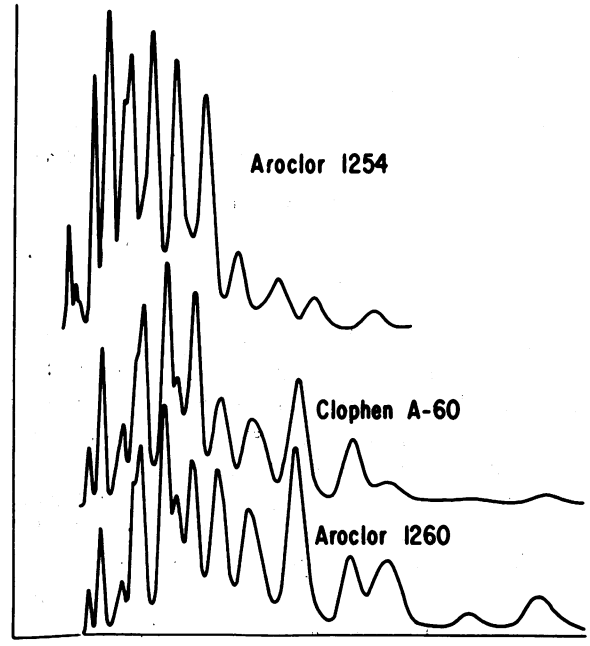


FIGURE 8. Comparison of gas chromatograms of Aroclor, 1254, Chlophen A-60 (Germany) and Aroclor 1260.

Figure 8 shows a comparison between the response of Aroclor 1260 and Clophen A-60 from Germany (2).

All the gas chromatograms shown above were produced utilizing an electron capture detector. This is an extremely good detector in many respects; however, care must be used in attempting to interpret data from it. First, it is not specific for any given type of compounds. It is generally sensitive to halogenated compounds, but many nonhalogenated ones also respond. Equally important, the electron capture response of halogenated compounds is not always in relation to the amount of chlorine. For instance, the response to carbon tetrachloride is 2,000 times greater than to ethylene dichloride—that is four chlorines in one case, and two in the other. This is an extreme example, but it points out the need for caution in interpreting response from unknown peaks. That is, a peak may or may not represent a halogenated compound, and it may represent a large or small amount. To be certain that any peak is a chlorinated compound, confirmatory tests are required. Using electron capture, amounts can only be estimated by comparison to the response of a known amount of the exact same compound.

The parameters of operating an electron capture detector also influence the responses. For instance, the voltage applied to the detector affects the relative responses between peaks.

Table 1 shows the relative responses of some individual tetrachlorobiphenyls to an electron capture detector (3).

Table 1. Retention time and electron-capture detector response of chlorobiphenyls

Compound	Relative retention time (p,p'-DDE = 1.00)	Relative response per ng ± standard deviation (p,p'-DDE = 1.00)
2,2',4,4'-tetrachlorobiphenyl	0.51	0.206±0.003
3,3',4,4'-tetrachlorobiphenyl	0.96	0.770±0.027
2,2',6,6'-tetrachlorobiphenyl	0.33	0.0403±0.0016
3,3',5,5'-tetrachlorobiphenyl	0.70	0.625±0.045
2,3,4,5-tetrachlorobiphenyl	0.69	0.715±0.010
2,3,5,6-tetrachlorobiphenyl	0.51	0.505±0.010
2,3,4,5,6-pentachlorobiphenyl	1.00	1.30±0.017

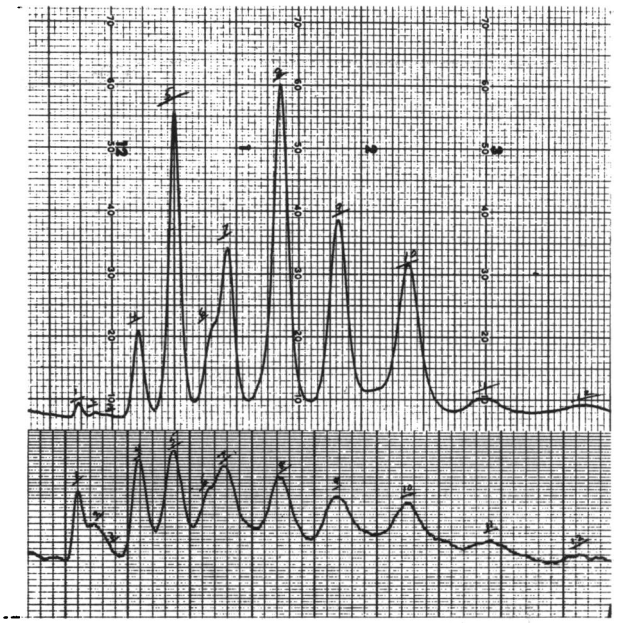


FIGURE 9. A comparison of response of 1254 utilizing different detectors. The upper curve is electron capture; lower curve is electrolytic conductivity detector.

Figure 9 shows the relative response from 1254 utilizing the same gas chromatographic conditions but different detectors; the upper curve is electron

Table 2. Physical properties of chlorobiphenyls

Compound	Melting point, C	Boiling point, C (mm Hg)
*2-chloro-	34	267–268; 154(12)
3-chloro-	89	284–285
*4-chloro-	76	125(14)
*2,2'-dichloro-	61	
3,3'-dichloro-	29	322–324; 320–326
*4,4'-dichloro-	148	315–319
3,5-dichloro-	36	166(10)
2,5-dichloro-		171(15); 182(30)
3,4-dichloro-	49	195–200(15)
2,3-dichloro-		172(30)
*2,4'-dichloro-	44	
3,4,3',4'-tetrachloro-	172	230(50)
3,4,2',5'-tetrachloro-	103	
2,6,2',6'-tetrachloro-	198	
*2,5,3',5'-tetrachloro-	162	
2,4,2',4'-tetrachloro-	83	
*2,5,2',5'-tetrachloro-	85	
2,4,5,3',4'-pentachloro-	179	195–220(10)
2,3,4,5,2',4',5'-heptachloro-		240–280(20)

* Present in commercial mixtures.
Reference (except *)—Hubbard.

capture, and the lower is the electrolytic conductivity detector. The latter is more nearly responsive to the amount of chlorine; therefore, it should more nearly represent the relative amount of the various components.

Use of a microcoulometric detector may also provide a better quantitative estimate. Coulometry should yield results which would permit an exact measure of the chlorine. However, the currently available microcoulometric equipment does not give strictly quantitative response. Both microcoulometry and electrolytic conductivity are more specific for halogen-containing compounds than the electron capture detector, and accordingly they can be used as a means to be more certain that the products detected contain chlorine.

Not enough is known about the chemistry of the individual components and their relation to the whole industrial product.

Some of the individual compounds are shown in Table 2 (1). It can be seen that the physical properties vary quite widely. Note the melting points of all the compounds listed; they are solids at room temperature. Yet when the commercial products are manufactured by chlorination of biphenyl, the mixtures formed vary from liquid to soft, sticky noncrystalline products with a chlorine content of 60%, low-melting resinous

Table 3. The retention indices, chlorine numbers and structures of the major PCB constituents in Aroclor 1254. (4)

Peak No.	R.I.	Chlorine No.[a]	NMR determined structure	Alternative predictions	
				Structure	R.I.[b]
22	1994	4	2,5-2′,5′	2-2′,3′,5′	1996
23	2010	4		2-2′,4′,5′	2012
				2,4-2′,5′	2010
24	2022	4	2,3-2′,5′	3-2′,4′,6′	2021
				2,4-2′,4′	2027
		(5)		2,6-2′,3′,6′	2017
29	2089	5	2,5-2′,3′,6′	2,6-2′,3′,5′	2092
32	2119	5	2,3-2′,3′,6′		
33	2136	4	2,5-3′,4′		
36	2159	5		2,5-2′,3′,5′	2159
39	2175	5	2,5-2′,4′,5′	2,4-2′,3′,5′	2175
				3,5-2′,4′,6′	2175
		(6)		2,6-2′,3′,4′,6′	(2175)
				2,3,6-2′,3′,6′	2172
41	2191	5	2,4-2′,4′,5′	2,3-2′,3′,5′	2189
42	2203	5	2,3-2′,4′,5′		
43	2207	5	2,5-2′,3′,4′		
44	2228	5	3,4-2′,3′,6′	2,4-2′,3′,4′	2226
45	2238	—		2,3-2′,3′,4′	2240
				2,6-3′,4′,5′	2240
				3,4-3′,5′	2238
				2,3,5-2′,4′,6′	2240
48	2264	6	2,3,6-2′,4′,5′	2,4-2′,3′,4′,6′	(2263)
50	2299	6	2,3,4-2′,3′,6′		
52	2321	5	3,4-2′,4′,5′		
55	2356	5	3,4-2′,3′,4′		
56	2356	6	2,4,5-2′,4′,5′	2,5-2′,3′,4′,5′	(2360)
				3,5-2′,3′,4′,6′	(2357)
59	2390	6	2,3,4-2′,4′,5′	2,3-2′,3′,4′,5′	(3291)
				2,4,6-3′,4′,5′	2388
		(7)		2,3,5-2′,3′,5′,6′	(2390)

[a] Cl No. between brackets; Chlorine number of minor constituent not associated with accurate R.I.
[b] R.I. between brackets: Predicted R.I. using Table VIII of Ref 4a.

products containing between 60 and 65% chlorine, and partly crystalline or crystalline products of much higher melting points containing more than 65% chlorine. Thus, the commercially-made mixtures have at least some physical properties that are quite different from any of the individual constituents. This is probably one of the very important factors in the industrial utility of the products. The same phenomenon probably has a bearing on the survival of many of the compounds through a host of possible conditions through the ecosystems. So it is remarkable that the end products in food, which we will see later, look as much like the original industrial product as they do.

A number of workers have identified the predominant number of chlorine atoms in the major components of some of the peaks. Others have made significant approaches to determine or predict the placement of the chlorines on the biphenyl rings (4), (5), (6).

Sissons and Welti (4) used a SCOT column and a flame ionization detector to obtain the graph shown in Fig. 10 from 1254. Note that there are 69 identifiable peaks. They did further purification, and then by utilizing MS, NMR, and factors such as retention time, they identified the major peaks of 1254 as shown in Table 3.

Note that the retention times do not necessarily relate to number of chlorines. These researchers also made use of retention times and other information to predict the composition of a large number of the other peaks. To attempt to be certain of the structure of the minor peaks would be tremendously complicated. It takes a significant amount of a chemical to make NMR analysis. It would be of great value to have available simple biological tests that would give a clue as to which peaks, major or minor, would be of enough significance to warrant full-scale chemical- and biological-effect investigations.

If it were found that all peaks contributed essentially equally to the biological effects (and there are certainly many biological effects), then there would be no need to perform a great amount of chemistry. On the other hand, if we determine that they vary in biological effects as widely as their propensity to capture electrons, then we would have to devise means to and measure these individual chemicals.

It is known that PCBs can be dechlorinated and oxidized by photochemical effects (7). I look forward to the discussions by Dr. Hutzinger on this subject. I want only to point out that these reactions may complicate the process of evaluation of both chemistry and toxicology. If the dechlorination is simply a reversal of chlorination, so that the higher chlorinated products revert to exactly the same compound in the lower chlorinated products, then the problem is not more complicated. If new and different chemicals form, then the problem is greater.

If there is significant photo–oxidation through the food chain, then we have the potential for chlorophenols and even the potential for the formation of polychlorodibenzofurans as further chemicals to evaluate. I hope that we hear discussion on these chemical problems during this conference.

Man has been exposed to residues of PCBs in his food in many forms; that is, from the original industrial product introduced accidentally into his food to cases where these compounds have been subjected to almost all conditions of food chain reactions, photochemical reactions, etc. Some of these conditions are:

A. PCB directly into food
   in rice oil (Japan)
B. PCB in paperboard
   transfers to food
C. PCB into animal food
   we eat animal tissue
D. PCB into environment
   into fish
   we eat fish
E. PCB into environment
   into fish
   fish into animal feed
   we eat animal tissue

The first example is that of PCB accidentally put into rice oil. The product consumed by man may have all the ingredients formed during the manufacturing process of the PCBs, plus any reaction that may be induced by food processing.

In the second example the food will have only those components which migrate from the packaging material to the food, either through the vapor phase or by contact, or both. If any polar compounds are present, they may not migrate so

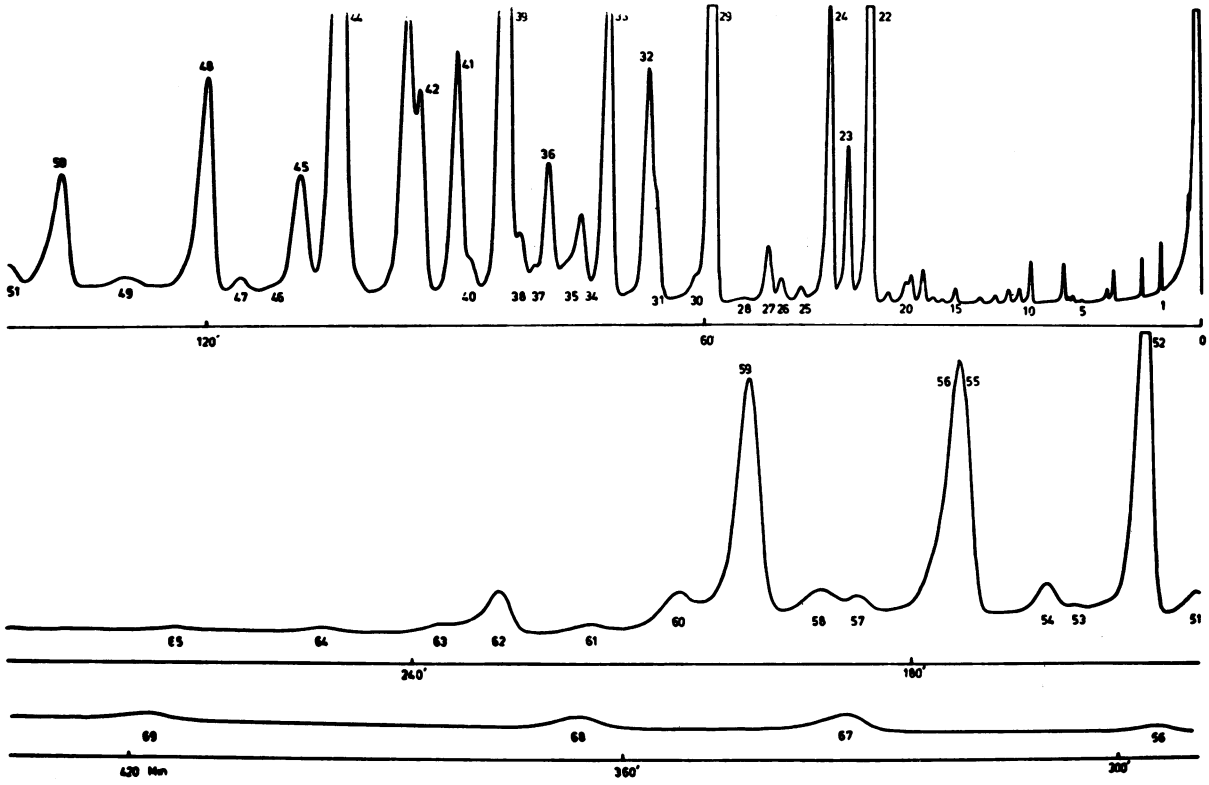


FIGURE 10. Aroclor 1254 solution chromatographed on an Apiezon L SCOT column at 205° employing a flame ionization detector (4).

readily. If the packaging material contains fat or wax, some of the higher chlorine compounds may not transfer as readily as the lower ones. However, there is the mutual effect of all on each, discussed earlier, which makes it difficult to predict. Some controlled experiments should be done.

In the third situation the whole industrial product contaminates the animal feed, and we eat the animal tissue. The animal appears to modify the product, because the gas chromatograms from tissue extracts do not look exactly like the industrial product.

In the fourth example the product or products have gotten into the environment, then into fish or other marine products, and we eat the fish or marine product. Here, there could be an accumulation of a number of different Aroclors or similar products, each of which has been subjected to various biochemical reactions through the food chain and to various other effects such as photochemical reactions. When we eat the fish, we eat the PCBs or metabolites, the end products of whatever reactions may have taken place.

And in the last example as in the previous one, the fish is fed to a domestic food animal—we eat the animal tissue.

The residues that we find in food products may look a good deal like the original industrial products or like a mixture of those products.

Figure 11 illustrates some food products, turkey and chicken tissues and cows' milk, which have been found to contain PCBs.

Figure 12 shows that the turkey sample had a product which looked most nearly like 1260. However, it can be seen that the proportion of peaks is different, and also a couple of peaks are missing.

Figure 13 shows that the chicken sample looked like 1248; yet there are some quite important differences.

Figure 14 shows some gas chromatograms from 1260 and the residues in muscle tissue from swine fed the 1260. Note that there are differences; they are not as great as in the case of the turkey tissue.

Figure 15 gives the results from a sample of coho salmon from Lake Michigan. A is before separation of pesticides from PCB, B is PCB after

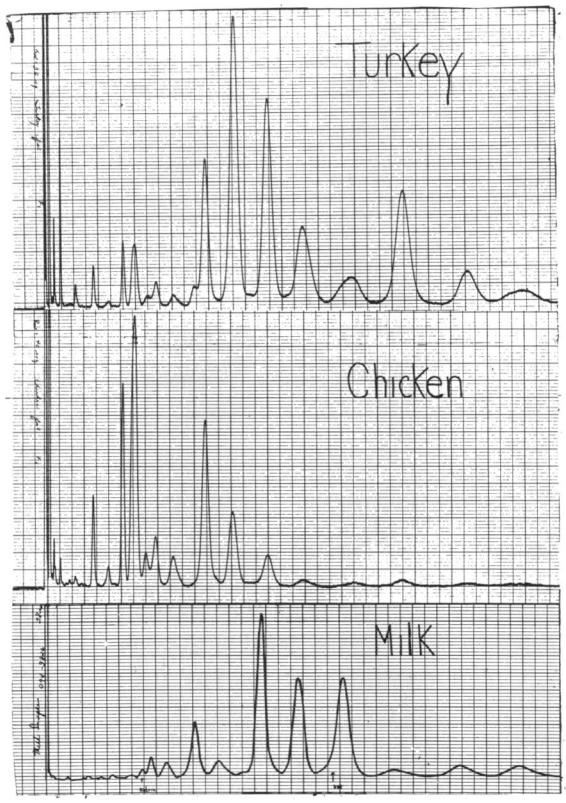


FIGURE 11. Gas chromatograms of PCBs from some food products.

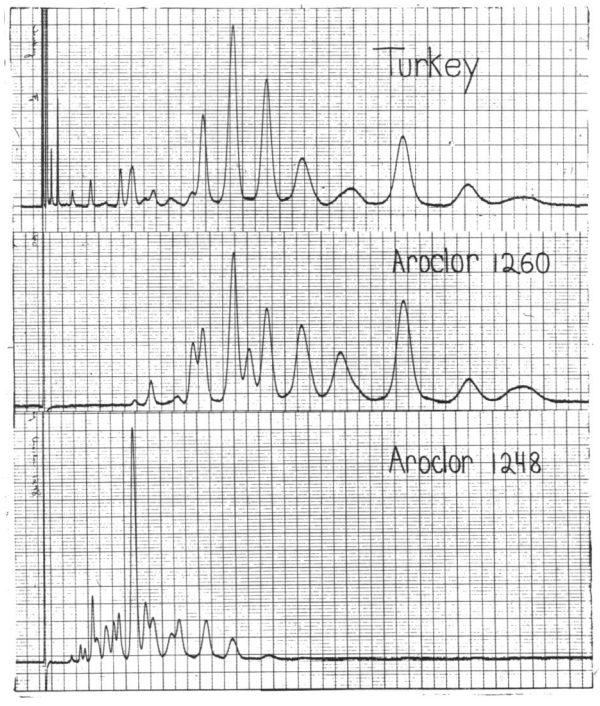


FIGURE 12. A comparison of gas chromatograms of PCB from turkey and Aroclor 1248 and 1260.

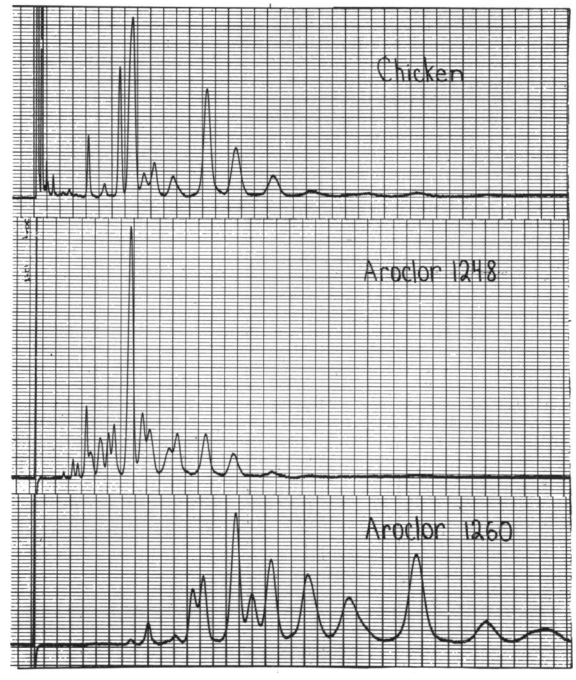


FIGURE 13. A comparison of gas chromatograms from chicken and Aroclor 1248 and 1260.

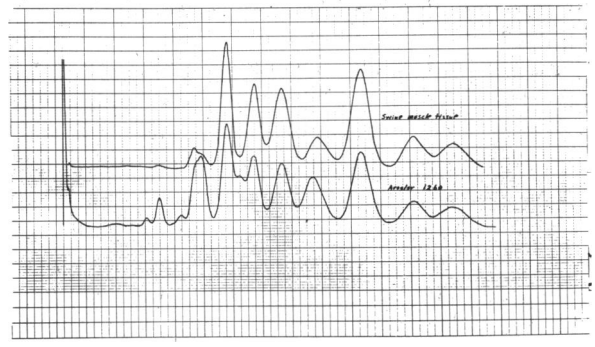


FIGURE 14. A comparison of gas chromatograms from Aroclor 1260 and residues in muscle tissue from swine fed Aroclor 1260.

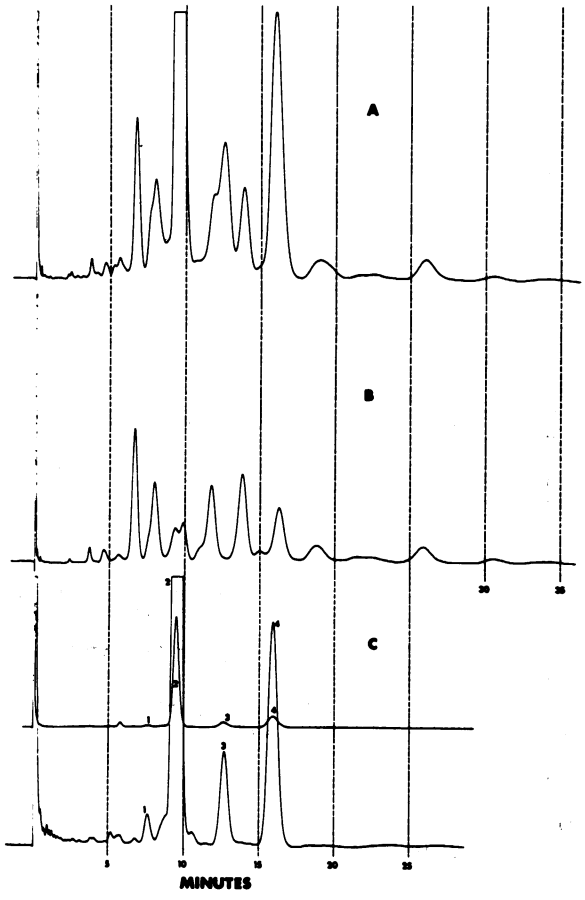


FIGURE 15. Gas chromatograms of sample from salmon from Lake Michigan. A is before separation of pesticides from PCB, B is PCB after separation and C is DDT pesticides.

separation, and C shows the DDT pesticides present. The PCBs here look most like 1254.

Many of the samples of wildlife reported by most workers yield chromatograms which look like 1254.

Figure 16 shows the residue in rat blood following the feeding of 1254. The peak, which disappeared, is a pentachloro compound. The material in the rat blood looks more like 1254 than does the material from coho salmon.

Veith (8) made a study of the PCBs in the Milwaukee River and out into Lake Michigan. He found that the lower chlorine content products disappeared more rapidly than the higher chlorinated ones. It may be that the residues in food products, which come from a long food chain, do

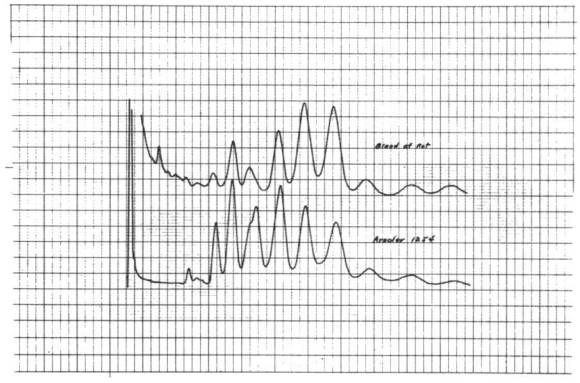


FIGURE 16. Comparison of gas chromatogram of Aroclor 1254 residue in rat blood following feeding of Aroclor 1254.

not originate entirely from the industrial product which is similar to the residue but may be the result of a concentration of the higher chlorinated units originally in the lower chlorine content products, the lower chlorinated units of which have largely disappeared.

So even though these industrial products are generally stable and persistent, they are altered by microorganisms, in animal systems, by light, and probably in plant systems. Some units disappear and some concentrate. We need to develop some short term in vitro and in vivo bioassay that can be used to give clues for more thorough and detailed examination. The chemist has tools and techniques to obtain almost any degree of sophistication required by the biologist.

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